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Estrogenic Potentials of Organochlorine Pesticides which Detected in Korean Adipose Tissue

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한국인의 지방조직에서 검출되는 유기염소제 농약의 에스트로겐 활성

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요 약

유기염소계 농약은 화학적으로 안정하고, 지용성이 크며 체내 대사에 대해 저항성을 가지고 있어서 인체 및 생태계의 생물체 중 축적성이 매우 크다. 또한 대부분 내분비계 장애물질로 분류되어 있어 관심의 대상이 되고 있다. 유기염소계 농약은 잔류성으로 인하여 사용이 금지되었음에도 불구하고 인체에서 검출되고 있으며 한국인의 지방조직을 분석한 결과 9종의 유기염소계 농약이 주로 축적되어 있음이 보고된 바 있다. 본 연구에서는 이들 유기염소계 농약의 에스트로겐 활성을 MCF-7 BUS cell을 이용한 E-screen assay competitive binding assay 및 pS_2 gene experession assay에 의해 조사하였다. *o,p'*-DDT, *p,p'*-DDT, *p,p'*-DDD, *p,p'*-DDE 등 4종의 유기염소계 농약은 에스트로겐 수용체에 대한 ligand 의존적인 작용기전에 의해 에스트로겐 활성을 가지며, α-, β-, γ-, δ-BHC, dieldrin 등 5종의 유기염소계 농약은 lingand-비의존적 작용기전에 의해 에스트로겐 활성을 보였다. 또한 이들 유기염소계 농약을 혼합 투여하여 에스트로겐 활성을 관찰한 결과 DDT류의 경우에는 단독투여시 보다 그 대사체와 혼합 투여할 때 에스트로겐 활성에 상승적 효과가 나타났으며 *o,p'*-와 *p,p'*-DDT의 두 이성질체를 혼합 투여할 경우가 단독 투여시 보다 상승적 효과가 나타났다. 따라서 지방조직에서 검출되는 유기염소계농약은 상호작용에 의해 개별 물질이 나타내는 내분비계장애작용 보다 실제로는 강한 효과를 나타낼 것으로 추정되었다.

Key words : organochlorine pesticide, estrogenicity, E-screen assay

INTRODUCTIONS

There has been increasing public concern that chemicals in the environment are affecting human health by disrupting normal endocrine function, particularly through interaction directly with steroid hormone receptors to alter endocrine functions. Environmental estrogens may be responsible for lowering the quantity and quality of human semen during the last 50 years and increasing the incidences of testicular cancer and cryptorchidism in men and

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breast cancer in women and men in industrialized countries (Sharpe *et al.*, 1993; Davis, 1993). Especially, many organochlorine pesticides (OCPs) possess hormonal activity and have thus been classified as endocrine disruptors.

OCPs were used worldwide throughout the United States and Europe from just after World War II through the 1970s. The use of DDT in the USA was banned in 1972 following the decline of many passerine species and impaired reproduction in raptors due to eggshell thinning (Lundholm, 1997). But, it is still being used for mosquito control in many developing countries (Wiktelius and Edwards, 1997). In Korea, a use of DDT had been prohibited in 1979.

OCPs are very chemically stable, lipophilic and resistant to metabolism, explaining its persistence and accumulation in the environment and organisms (ATSDR, 1989). Due to their lipophilic property, OCPs more tend to accumulate in adipose tissue and are detected in Korean environment. DDTs concentration in Korean adipose varied between 0.0098 and 0.8987 μg/g on lipid weight basis. BHCs concentration in Korean adipose varied between ND and 0.2160 μg/g on lipid weight basis. Dieldrin concentration in Korean adipose varied between ND and 0.0052 μg/g on lipid weight basis (Yoo *et al.*, 2001; Yoo *et al.*, 2002). OCPs are also detected in Korean environment, e.g. 2.26 ng/g (dry weight) in the Lake Shihwa and 12.6 ng/g (dry weight) in the Masan Bay (Jong *et al.*, 1999; Khim *et al.*, 1999).

In this study, we examined estrogenic potentials induced by treatment with single and combination of OCPs (DDT, DDD, DDE, BHC, and dieldrin), which were accumulated in Korean human adipose tissue. To explore estrogenic activity of these compounds, cell proliferation, whole cell competitive ER binding and pS_2 mRNA expression in MCF7-BUS cell were examined.

MATERIALS AND METHODS

1) Cell lines

In this study, MCF7-BUS cell lines was used in all method. Human breast cancer estrogen sensitive MCF7-BUS cell lines were a kind gift of Dr. Soto (Tufts university, Bostone, USA). Cells were grown in Dulbecco's modification of Eagle's medium (DMEM) supplemented with 5% fetal bovine serum (FBS), penicillin (10,000 units/ml) and streptomycin (10,000 μg/ml) at 37°C in an atmosphere of 5% CO_2/95% air under saturating humidity.

2) Stripping sex steroids by Charcoal-Dextran treatment from Serum

Charcoal was washed twice with cold sterile water immediately before using. A 5% charcoal-0.5% dextran T70 suspension was prepared. Charcoal-dextran (CD) suspensions were centrifuged at 2,000 ×g for 10min. Supernatants were aspirated and serum aliquots were mixed with the charcoal pellets. This charcoal-serum mixture was maintained in suspension by rolling at 6 cycles/min at 37°C for 1 hr. This suspension was centrifuged at 2,000 ×g for 20min. The supernatant was filtered through a 0.45-μm and 0.22-μm Nalgene filter. CD-FBS was stored at -20°C until needed.

3) The cell proliferation assay

These assay, one of which is referred to as the E-Screen, use ER-positive MCF-7 human breast cancer cells. MCF-7 is stable cell line derived from a metastatic adenocarcinoma of the human breast that is a widely used model of estrogen-dependent cell proliferation. The growth of MCF-7 cells *in vitro* is stimulated by estrogens and inhibited by antiestrogens (Spink *et al.*, 1990). Cloned MCF7-BUS cells were trypsinized and seeded into 48-well plates at initial concentrations of 5,000 cells per well. The cells were attached for 48 hrs, the seeding medium (5% FBS in DMEM) was replaced by the experimental medium (10% CD-FBS supplemented to phenol red-free DMEM). Test compounds were added to this medium with a range of concentration. The bioassay was terminated in 144 hrs by removing media from the well, fixing the cells and staining with sulforhodamine-B (SRB). The fixation and staining technique was modified from that described by Skehan *et*

al (1990). Briefly, the cells were treated with cold 10% trichloroacetic acid and incubated at 4°C for 30 min. Then the cells were washed five times with tap water and left to dry. The fixed cells were stained for 10 min with 0.4% (w/v) SRB dissolved in 1% acetic acid. Wells rinsed five times with 1% acetic acid and air dried. Bound dye was solubilized with 10 mM Tris base (pH 10.5) in a shaker. Optical density was measured at 490 nm using a microplate reader (Molecular Device, Versa Max. USA).

4) pS_2 mRNA expression assay (RT-PCR)

The pS_2 (a trefoil peptide expressed in breast cancer cells) gene is an endogenous gene regulated by estrogen and serves as a marker for the ER positive tumors (Dante *et al.*, 1994; Kalanithi *et al.*, 2000). The reason we used the expression of pS_2 as markers of estrogenic effects is based on pS_2 is an estrogen induced gene product first identified in the MCF-7 human breast cancer cell line after estrogen treatment, but no found in normal breast tissue nor in any other cultured human cell lines (Lifen *et al.*, 1997). Its expression was studied in MCF7-BUS cells. MCF7-BUS cells suspended in DMEM (5% FBS) were seeded into 60 mm dish at a density of 1,000,000 cells/5 mL per dish. Cells were attached for 48 hrs, the seeding medium was replaced by the experimental medium (3% CD-FBS supplemented to phenol red-free DMEM). After 24 hrs, the medium changed to DMEM (3% CD-FBS supplemented to phenol red-free DMEM) containing test chemicals. The cells were incubated at 37°C and 5% CO_2 for 1 day. Thereafter the cells were harvested for the extraction of RNA. The total RNA was purified with 1 mL of Trizol-Reagent (GIBCO), phase-separated with chloroform and precipitated using isopropanol. The RNA was washed with 75% ethanol, then dissolved in diethyl pyrocarbonate-treated water and stored at −80°C. The RNA was quantified by measuring the absorbance at 260 nm with spectrophotometer. According to the protocol of the RT-PCR kit (promega A1250 kit), RNA samples (1 μg) were reverse transcribed into cDNA using 3'-primer and the transcribed cDNA was amplified by PCR thermocycler (Perkin Elmer) using 5'- and 3'-primers. The thermal cycle profile used in this study was 1 cycle at 94°C for 2 min, 25 cycles of 1) denaturation a 94°C for 30 sec, 2) annealing at 60°C for 1 min, 3) extension at 68°C for 2 min, and 1 cycle at 68°C for 7 min. A portion (5 μl) of the PCR mixture was electrophoresed in 1.5% agarose gel in TBE buffer. The gel was stained with ethidium bromide. PCR products were detected and analyzed with Gel documentation & analysis system (UVP). The cDNA sequences of human pS_2 and α-actin are available in the GenBank. The primers used are :

(1) pS_2-up: 5'-CATGGAGAACAAGGTGATCTG-3'
(2) pS_2-down : 5'-CAGAAGCGTGTCTGAGGTGTC-3'
(3) α-actin-up : 5'-GGAGCAATGATCTTGATCTT-3'
(4) α-actin-down : 5'-CCTTCCTGGGCATGGAGTCCT-3'.

The PCR product for α-actin is 204 bp long, 336 bp for pS_2 (Lifen *et al.*, 1997).

5) Whole cell competitive estrogen-receptor binding assay

The whole cell competitive estrogen-receptor binding assay was similar to the Gierthy *et al.* (1996). MCF7-BUS cells suspended in DMEM (5% FBS) were seeded into 24-well plates at a density of 500,000 cells/mL per well. Cells were attached for 48 hrs, the seeding medium was replaced by the experimental medium (3% CDFBS supplemented to phenol red-free DMEM). 24 hrs later, medium was changed to DMEM (3% CDFBS supplemented to phenol red-free DMEM) containing 1 nM [2, 4, 6, 7-^{3}H] E_2 alone or in combination with unlabeled E_2 (from 10^{-13} M to 10^{-8} M) or test compounds (from 10^{-9} M to 10^{-5} M), and incubated at 37°C for 3 hrs. After the incubation, the culture medium was removed and the wells were washed five times with ice-cold PBS. The washed cells were dissolved in 1 mL of 10 mM EDTA (pH 12.4) and aliquots were then taken to scintillation counting vial. The radioactivity in 1 mL

of the EDTA extract solution was determined by scintillation counting. The amount of bound [2, 4, 6, 7-^{3}H]-E_2 in the presence or absence of the test compound was calculated after correcting for non-specific binding as measured by the amount of bound [2, 4, 6, 7-^{3}H]-E_2 in the presence of 2,000-fold excess E_2. Data are expressed as the ratio of bound [2, 4, 6, 7-^{3}H]-E_2 in the presence of a competitor to the bound [2, 4, 6, 7-^{3}H]-E_2 in control (0.1% ethanol) medium, $\times$ 100 (Gierthy *et al.*, 1996).

6) Statistical analysis

The results were expressed as the mean$\pm$SD. In the E-screen assay, cell proliferation yield experiments conducted in quadruplicate wells were repeated at least a minimum of three times. In the whole cell competitive ER binding assay, [2, 4, 6, 7-^{3}H]-E_2 binding experiments conducted in triplicate wells were repeated at least a minimum of three times. In this study, statistical significance was determined by the Student's t-test. A p value of ≤ 0.05 was regarded significant. In the E-screen assay of OCPs combination, statistical significance of treatment groups were determined by the Duncan multiple range test. A p value of ≤ 0.05 was regarded significant.

RESULTS AND DISCUSSIONS

1. ER binding affinity of organochlorine esticide

Since the effects of estrogen are mediated by binding to its intracellular receptor (E domain), we tested the binding affinity of 17β-estradiol and OCPs to ER in the whole cell competitive ER binding assay. The binding affinity of 17β-estradiol, from 10^{-13} M to 10^{-8} M concentrations, was tested in the whole cell competitive ER binding assay, this result is shown in Fig. 1. 17β-Estradiol competed with [2, 4, 6, 7-^{3}H] E_2 for binding to the estrogen receptor in a dose-dependent manner. The dose-dependent response of OCPs in whole cell competitive ER binding assay is shown in Fig. 1. *o, p'*-DDT, *p, p'*-DDT, *p, p'*-DDD and *p, p'*-DDE competed with [2, 4, 6, 7-^{3}H] E_2 for binding to the estrogen receptor, it means that these compounds have ER binding affinity (Fig. 1A). But the others did not competed with [2, 4, 6, 7-^{3}H] E_2 for binding to the estrogen receptor even at a concentration that was 10,000-fold higher than the [2, 4, 6, 7-^{3}H] E_2, it means that these compounds have no ER binding

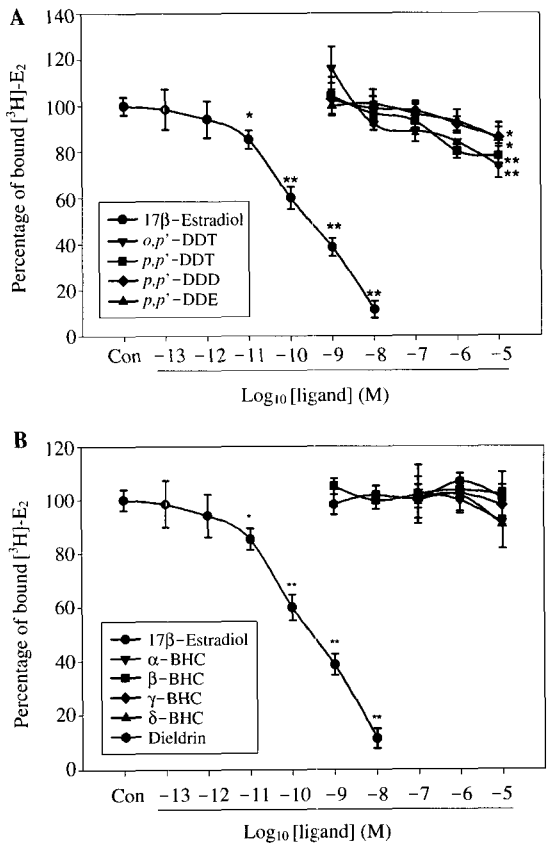
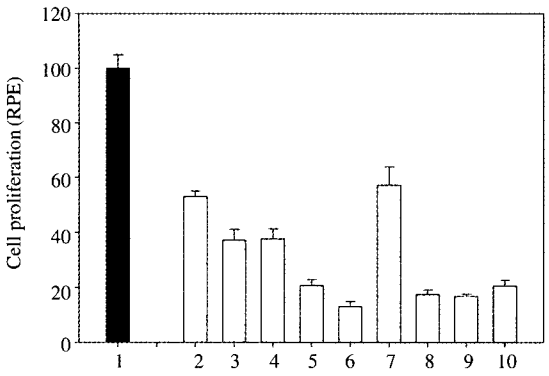


Fig. 1. Displacement of [^{3}H]-E_2 by chemicals in the whole cell competitive ER binding assay using MCF7-BUS cell lines at 37°C, 3 hrs incubation. Unbound radioligand was removed as described in Methods, and specific bound radioligand was calculated by subtracting nonspecific bound counts from total bound counts. Results are expressed as the means and standard deviation of three measurements for each data point. Significantly different from vehicle-control group (100%, $^*p<0.05$, $^{**}p<0.01$)



	Compounds	RPE (%)
1	17β-Estradiol	100.00±4.97
2	*o, p'*-DDT	52.95±2.17
3	*p, p'*-DDT	37.18±4.05
4	*p, p'*-DDD	37.64±3.70
5	*p, p'*-DDE	20.67±2.17
6	α-BHC	12.87±1.92
7	β-BHC	57.32±6.71
8	γ-BHC	17.41±1.69
9	δ-BHC	16.74±0.92
10	Dieldrin	20.57±2.02

Fig. 2. Effects of E_2 (10^{-10} M), organochlorine pesticides (10^{-5} M) treatment on the growth of MCF7-BUS cells in culture. Cells were plated in 48-well plate at a cell density 5,000 cells/well and allowed to attach for 48 hrs. Cells were incubated in DMEM supplemented with 10% CDFBS with test compounds for 144 hrs. Incubation with ethanol alone was performed as control and the final concentration of ethanol in the medium never exceeded 0.1%. After incubating for 144 hrs, SRB assay was conducted to measure cell proliferation. SRB assay was conducted as described in Methods. The proliferative effect of compounds relative to E_2 (10^{-2}M, 100%) represent as RPE (Relative Proliferative Effect). Results are expressed as means and standard deviation of three separate experiments for each data point. (1) 17β-Estradiol; (2) *o, p'*-DDT; (3) *p, p'*-DDT; (4) *p, p'*-DDD; (5) *p, p'*-DDE; (6) α-BHC; (7) β-BHC; (8) γ-BHC; (9) δ-BHC; (10) Dieldrin

affinity (Fig. 1B).

2. Estrogenicity of single organochlorine pesticide

The E-SCRREN assay is based on the estrogen-receptor binding induced proliferation of the human breast cancer cell line, MCF7-BUS. In E-SCREEN assay, maximum cell proliferative effect of compounds relative to E_2 (10^{-10}M, 100%) represented as RPE (Relative Proliferative Effect).

The estrogenicity of *o, p'*-DDT, *p, p'*-DDT, *p, p'*-DDD, *p, p'*-DDE, α-, β-, γ-, δ-BHC and dieldrin were determined by E-screen assay. Cell proliferation of single OCPs (10^{-5} M) is shown in Fig. 2 and RPE of each OCP is summarized. The values of RPE mean that nine OCPs are partial agonists(Ob *et al.*, 2000) RPE of OCPs was in order of β-BHC followed by *o, p'*-DDT > *p, p'*-DDD > *p, p'*-DDT > *p, p'*-DDE > Dieldrin > γ-BHC > δ-BHC > α-BHC.

To determine whether the observed stimulatory effects of OCPs on cell proliferation were mediated by estrogen receptor, estrogen antagonist tamoxifen (TM) was co-treated with OCPs in MCF7-BUS. In MCF-7 cell cultures, tamoxifen does not control replication produced by epidermal growth factor, which promotes DNA synthesis independent of estrogen activity or its affinity to ER (Cormier *et al.*, 1989). Cell proliferations of all OCPs were significantly inhibited by addition of the TM ($p<0.01$ as determined by the Students' *t*-test) except α-BHC ($p<0.05$ as determined by the Students' *t*-test). Cell proliferations of *p, p'*-DDT, *p, p'*-DDD, *p, p'*-DDE, β-BHC, γ-BHC, δ-BHC and dieldrin were completely blocked by addition of TM (10^{-6} M) (Fig. 3). Therefore, it was suggested that cell proliferation induced by these chemicals were mediated through the ER-dependent pathway. However, cell growth of *o, p'*-DDT and α-BHC was partly inhibited by addition of the TM. Therefore, it was demonstrated that cell proliferation induced by these chemicals were mediated through ER-independent pathway as well as ER-dependent pathway.

To test whether OCPs (10^{-5} M) can regulate expression of an endogenous estrogen-regulated gene, expression of pS_2 was examined. As shown in Fig. 4,

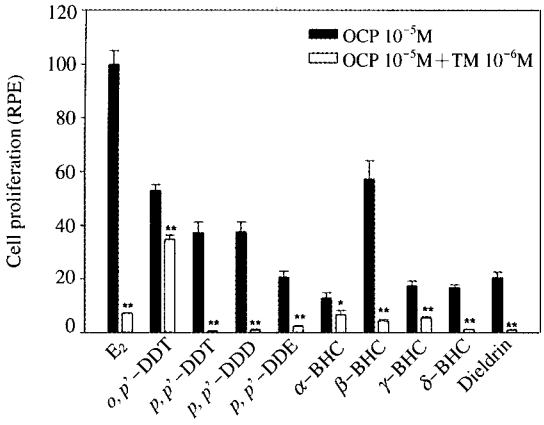


Fig. 3. Effects of cotreatment with antiestrogen (Tamoxifen) on cell proliferation induced by organochlorine pesticides (OCP; 10^{-5} M) in MCF7-BUS cells. Cells were plated in 48-well plate at a cell density 5,000 cells/well and allowed to attach for 48 hrs. Cells were incubated in DMEM supplemented with 10% CDFBS with test compounds for 144 hrs. Incubation with ethanol alone was performed as control and the final concentration of ethanol in the medium never exceeded 0.1%. After incubating for 144 hrs, SRB assay was conducted to measure cell proliferation. SRB assay was conducted as described in Methods. The proliferative effect of compounds relative to E_2 (10^{-10}M, 100%) represent as RPE (Relative Proliferative Effect). Results are expressed as means and standard deviation of three separate experiments for each data point. Comparisons were made with Student's tests. Values significantly different from each group (E_2 or each OCP: *$p<0.05$, **$p<0.01$).

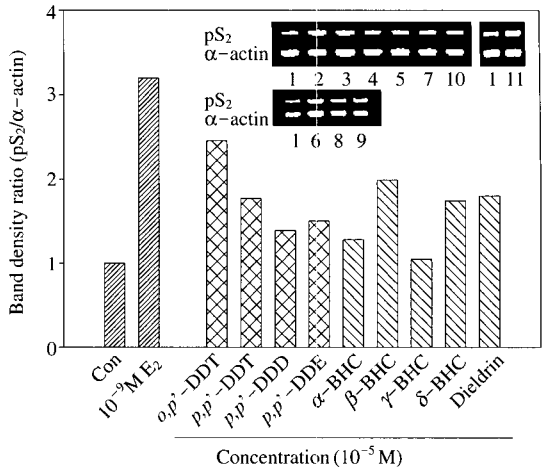


Fig. 4. Effects of OCP (10^{-5} M) treatment on pS_2 mRNA expression in MCF7-BUS cells in culture. Cells were plated in 6-well plate at a cell density 50,000 cells/well and allowed to attach for 24 hrs. To deplete steroid in cells, cells were incubated with phenol red-free 3% CDFBS-DMEM for 24 hrs. After 24 incubations, the cells were incubated in DMEM supplemented with free steroid CDFBS 10% with combination of E_2 and kaempferol for 48 hrs. Ethanol was used as negative control (Con), and E_2 was used as a positive control. The final concentration of ethanol in the medium never exceeded 0.1%. After the cell incubated for 48 hrs, total RNA was extracted using TRIzol reagent (Gibco BRL). pS_2 mRNA levels were measured by RT-PCR and normalized using α-actin mRNA as an internal standard. RT-PCR was conducted as described in Methods. 1 μg total RNA were applied to RT-PCR reaction for 25 cycle with pS_2 and α-actin primers. RT-PCR products were run on an ethidium bromide stained 1.5% agarose gel, which was then scanned with the Gel documentation & Analysis system. 1, EtOH; 2, *o,p'*-DDT; 3, *p,p'*-DDT; 4, *p,p'*-DDD; 5, *p,p'*-DDE; 6, α-BHC; 7, β-BHC; 8, γ-BHC; 9, δ-BHC; 10, Dieldrin; 11, E_2

β-BHC, *o, p'*-DDT, *p, p'*-DDT, δ-BHC and dieldrin found to be estrogenic in the pS_2 mRNA expression assay similar to E-screen assay. However, BHCs and dieldrin did not show binding affinity to ER the ER (Fig. 1). Therefore, it was suggested that estrogenic activity of β-BHC, δ-BHC and dieldrin was not induced through classical estrogen response pathway via the estrogen receptor.

3. Estrogenicity of combined organochlorine pesticide

When contemplating the biologic activity of chemical mixture, it is necessary to consider that multiple xenobiotics may exhibit synergistic or antagonistic interactions rather than simple additive interactions (Douglas *et al.*, 2000). To study combined estrogenic activity of OCPs, the E-screen assay was conducted in 10^{-7} M and 10^{-6} M concentration of combined OCPs. 10^{-5} M concentration of combined OCPs was cytotoxic to MCF7-BUS cell line, and 10^{-9} M and

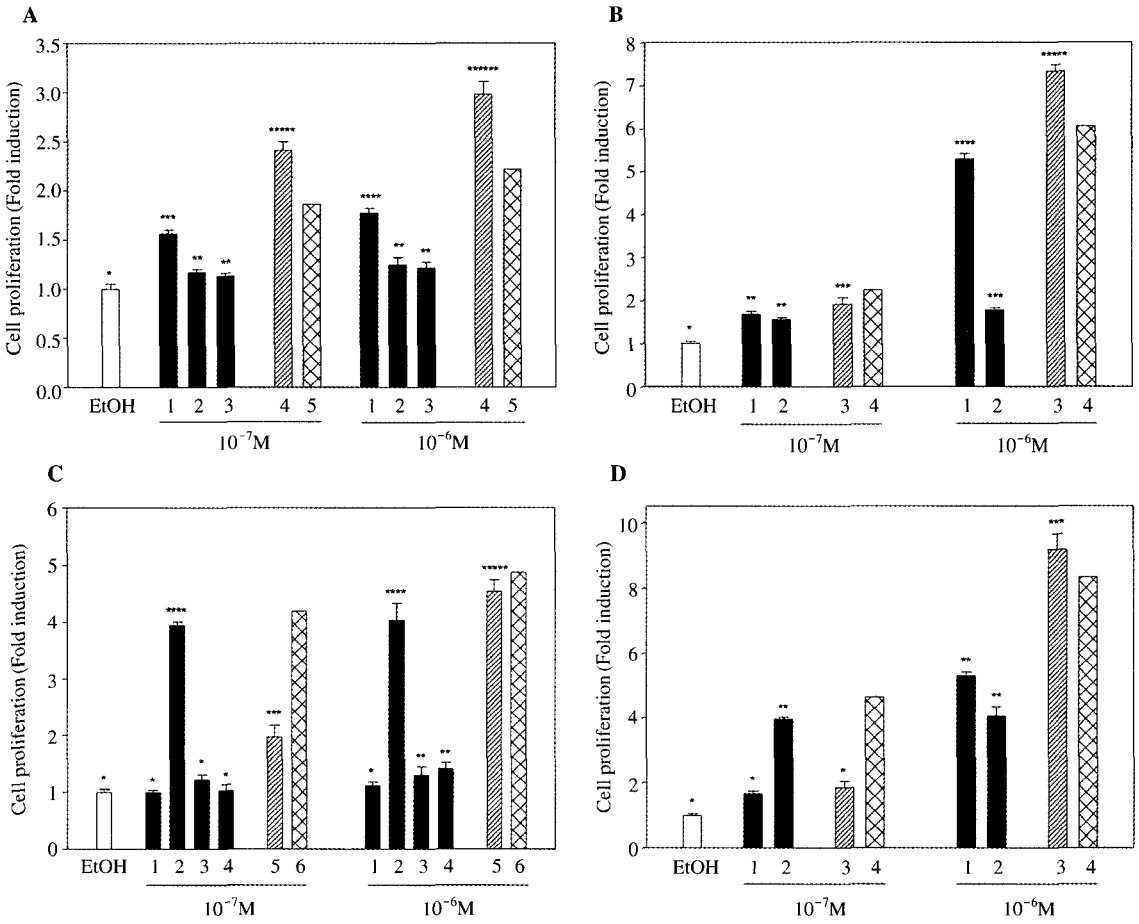


Fig. 5. Combination effects of OCPs (10^{-7} M and 10^{-6} M) treatment on the growth of MCF7-BUS cells in culture. Each point represents the mean and standard deviation of four measurements. Treatment groups with the same number of asterisks are not significantly different from each other. A: 1, *p, p'*-DDT; 2, *p, p'*-DDD; 3, *p, p'*-DDE; 4, 3-DDT; 5, Expected-3-DDT (*p, p'*-DDT+*p, p'*-DDD+*p, p'*-DDE), B: 1, *o, p'* -DDT; 2, *p, p'* -DDT; 3, 2-DDT; 4, Expected-2-DDT (*o, p'*-DDT+*p, p'*-DDT), C: 1, α-BHC; 2, β-BHC; 3, γ-BHC; 4, δ-BHC; 5, 4-BHC; 6, Expected 4-BHC (α-BHC+β-BHC+γ-BHC+δ-BHC), D: 1, *o, p'* -DDT; 2, β-BHC; 3, 2-OCP; 4, Expected-2-OCP (*o, p'* -DDT+β-BHC)

10^{-8} M concentration of combined OCPs had no effect in the E-screen assay.

The combined effect of OCPs was evaluated by comparing with experimental value and expected value. The expected estrogenic effect was calculated by addition of the response of the single compounds (Wofgang *et al.*, 1999). In addition to, significant difference of response to the single compounds and combined mixture was also seen by the Duncan multiple range test.

To evaluate combined estrogenic effect of *p, p'*-DDT and their metabolites, combined mixture of *p, p'*-DDT, *p, p'*-DDD and *p, p'*-DDE was made in equimolar concentrations and this combined mixture was expressed as 3-DDT. As shown in Fig. 5A, the experimental value of 3-DDT was greater than the expected value. This result suggested that the combined effect of *p, p'*-DDT and its metabolites was

synergistic.

Technical grade DDT contains 80 % of *p, p'*-DDT isomer and 20% of *o, p'*-DDT. And, among DDT isomers, *o, p'*-DDT was more potent than *p, p'*-DDT. The combined mixture of *o, p'*-DDT and *p, p'*-DDT made in equimolar concentrations and this combined mixture was expressed as 2-DDT. As shown in Fig. 5B, the experimental value of 10^{-7} M 2-DDT was smaller than the expected value, but this value is significantly greater than the experimental value of single OCPs by the Duncan multiple range test. The experimental value of 10^{-6} M 2-DDT was greater than the expected value. This result suggests that the combined effect of 10^{-6} M DDT isomers, not 10^{-7} M DDT isomers, was only synergistic.

The combined mixture of α-BHC, β-BHC, γ-BHC and δ-BHC was made in equimolar concentrations and this combined mixture was expressed as 4-BHC. As shown in Fig. 5C, the experimental value of 10^{-7} M 4-BHC was smaller than the expected value and this value was significantly smaller than the experimental value of single OCPs by the Duncan multiple range test. The experimental value of 10^{-6} M 4-BHC was smaller than the expected value but this value was significantly greater than the experimental value of single OCPs by the Duncan multiple range test. This result suggests that the combined effect of 10^{-6} M BHC isomers was additive effect, but not synergistic effect.

o, p'-DDT can act to estrogen response element by the classical ER pathway, on the other hand, β-BHC can act estrogen response element by the non-classical ER pathway. The estrogenic effect of two OCPs were significantly great in the E-screen assay and the pS_2 mRNA expression assay. The combined mixture of *o, p'* -DDT and β-BHC was made in equimolar concentrations and this combined mixture was expressed as 2-OCP. As shown in Fig. 5D, the experimental value of 10^{-7} M 2-OCP was smaller than the expected value and this value is significantly smaller than the experimental value of single OCPs by the Duncan multiple range test. The experimental value of 10^{-6} M 2-OCP was greater than the expected value. This result suggests that the combined effect of 10^{-7} M 2-OCP was antagonistic and the combined effect of 10^{-6} M 2-OCP was synergistic.

CONCLUSIONS

In this study, we evaluated estrogenicity and ER-mediated response of nine OCPs detected in Korean adipose tissue. According to previous report, *p, p'*-DDT, *p, p'*-DDD, *p, p'*-DDE, α-BHC, β-BHC, γ-BHC and dieldrin, which were already banned in 1970s, were detected in Korean abdominal adipose tissue by GC/MSD.

Nine OCPs which tested in this study stimulated cell proliferation in a dose-dependent manner in the ER-positive cell lines MCF7-BUS and these effects were inhibited by inclusion of the antiestrogen, TM. All OCPs increased the pS_2 mRNA level in the MCF7-BUS cell lines. On the other hand, we tested in the whole cell competitive ER binding assay, *o, p'*-DDT, *p, p'*-DDT, *p, p'*-DDD and *p, p'*-DDE displace [2, 4, 6, 7-^{3}H] E_2 from the ER, but, α-, β-, γ-, δ-BHC and dieldrin did not displace [2, 4, 6, 7-^{3}H] E_2 from the ER even at a concentration that was 10,000 fold higher than the tracer steroid. These indicates that the selected chemicals and their estrogenicity priority rankings are β-BHC, *o, p'*-DDT, *p, p'*-DDD, *p, p'*-DDT, *p, p'*-DDE, dieldrin, γ-BHC, δ-BHC and α-BHC. Especially, we suggested that *o, p'*-DDT, *p, p'*-DDT, *p, p'*-DDD and *p, p'*-DDE revealed the ligand-dependent ER activation mechanism, α-, β-, γ-, δ-BHC and dieldrin showed the ligand-independent ER activation mechanism. For example, β-BHC, unlike E_2, was known to activate a minimal promoter reporter gene containing a single ERE sequence but was as effective as E_2 in activating more complex estrogen-responsive genes. It is presumed that transcriptional activity of β-BHC may not require ERE sequences, but may actually be mediated through other response elements such as AP-1-binding sites (Rosemary *et al.*, 1996). The E-screen assay was sensitive to evaluate the estro-

genicity of OCPs.

In addition we evaluated mixture effects of OCPs' estrogenicity. It was observed that OCPs exhibited synergistic or antagonistic interactions rather than simple additive interactions. Therefore, it was suggested that the estrogenic action of persistent OCPs residues in adipose tissue may play a role in the endocrine disruption and the progression of hormonal responsive tumors of the breast and uterus.

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